OP29
ECHO: the executable chondrocyte

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Introduction: We recently presented ANIMO (Analysis of Networks with Interactive Modeling), a software tool for modeling dynamic molecular networks for use by biologists [1, 2]. We used ANIMO to generate a computational model of articular cartilage.
Materials and methods: Based on a large-scale literature study [3] and our own experiments, we developed ECHO (Executable Chondrocyte), a computational model of the key processes that regulate expression and activity of SOX9 and RUNX2, two master transcriptional regulators of the chondrocyte phenotype. ECHO consists of 93 nodes with 274 interactions that describe the expression and activity of 52 genes and proteins. Simulations in ECHO were performed to investigate the robustness of the chondrocyte network. To validate ECHO predictions, we used FRAP to measure mobility of SOX9 and RUNX2, which we have shown to be a faithful readout of their activity.
Results: In its unperturbed form, ECHO displays two stable states in which activities of SOX9 and RUNX2 are mutually exclusive. SOX9 represents a stable articular cartilage phenotype, while RUNX2 represents transient hypertrophic cartilage. We tested *in silico* the hypothesis that addition of WNT (performed with a few clicks of the mouse) will change permanent cartilage into transient cartilage by inducing hypertrophy. Indeed, when we add WNT, a known regulator of bone formation, the permanent or $SOX9^+$ state changes to a transient or $RUNX2^+$ state in the model. However, it is known that healthy articular cartilage is resistant to hypertrophic differentiation. Our group has previously shown experimentally that this was probably due to the secretion of DKK1, FRZB and GREM1[4, 5]. We therefore added nodes to ECHO representing DKK1, FRZB and GREM1 (fig. 1). GREM1 and DKK1 are able to stabilize the permanent cartilage or $SOX9^+$ state even after addition of WNT in ECHO.

We observed that in our model activation of WNT leads to a switch from a SOX9 + state to a RUNX2 + state. To prove that WNT/β-catenin signaling can directly regulate SOX9 function, we investigated the response of SOX9 mobility to WNT3A in live primary chondrocytes. Addition of WNT3A to human chondrocytes transfected with SOX9-GFP resulted in a significant decrease of the immobile SOX9 fraction from 53% to 34% within 15 minutes after addition, indicating a loss of transcriptional activity of SOX9.
Discussion and conclusion: Using ECHO we predicted the stimuli that prevent hypertrophic differentiation of articular cartilage, and tested this experimentally with FRAP using SOX9 and RUNX2 mobility as a read-out.

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Disclosures: The authors have nothing to disclose.

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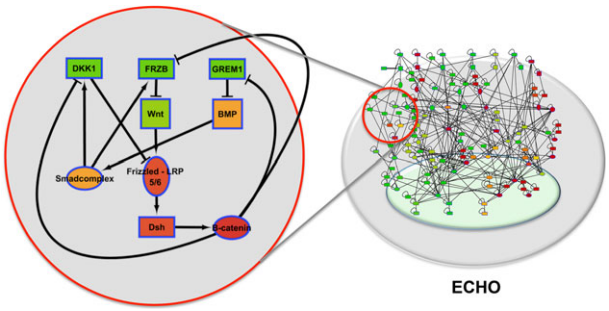

Figure 1 A complex network of many signal transduction pathways determines the development of either transient or permanent cartilage. We perform *in silico* experiments in the complete network, or in smaller areas.

OP30
ANIMO: a tool for modeling biological pathway dynamics

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Introduction: Computational methods are applied with increasing success to the analysis of complex biological systems. However, their adoption is sometimes made difficult by requiring prior knowledge about the foundations of such methods, which often come from a different branch of science.

The software ANIMO (Analysis of Networks with Interactive MOdeling, [1]) allows the tissue engineer to add dynamic behavior to traditional static models of signaling events. We use ANIMO to optimize cartilage tissue engineering.
Materials and methods: Starting from a signaling network as traditionally represented in books, ANIMO allows biologists to take advantage of their expertise, enriching the symbolic description with quantitative parameters. The underlying computational model is based on the formalism of Timed Automata [2] and is automatically generated and analyzed by ANIMO. Implementation as a Cytoscape [3] plug-in makes the interface intuitively usable: for example, an existing network topology can be extended with few mouse clicks, adding new nodes and

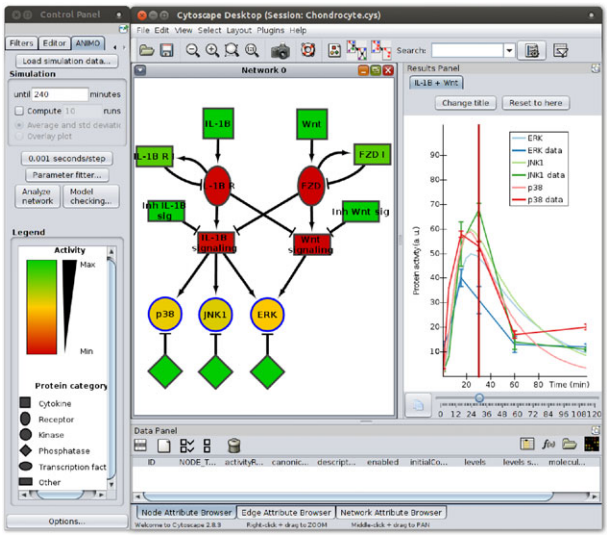

Figure 1 ANIMO user interface. The vertical red line in the graph on the right corresponds to the point in the simulation run on which coloring of nodes in the network is based.

edges. The use of ANIMO does not require detailed knowledge of the underlying formalism of the model.
Results: Figure 1 shows the user interface of Cytoscape enriched by ANIMO: simulation runs are shown in form of graphs (on the right). The course of a simulation is mirrored in the network (left) thanks to a user-movable slider under the corresponding graph: different colors indicate different activity levels as shown in the Legend. The Timed Automata model is automatically produced by ANIMO from the network defined in the Cytoscape interface, completely transparently to the user. The models can also be analyzed with the technique of model checking, thanks to the application of the powerful tool UPPAAL [4]. Also this analysis occurs behind the scenes, presenting the user with a querying interface based on human language.
Discussion and conclusions: The interactive nature of ANIMO combined with the modeling power of the underlying formalism allows the tissue engineer to create and explore executable models [5] of biological networks, helping to derive hypotheses and to plan wet-lab experiments in a familiar environment.

To validate ANIMO, we will present a small model of chondrocyte response to IL-1β and Wnt signaling, and show how ANIMO can optimize conditions for cartilage tissue engineering.
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OP31
Anti-inflammatory properties of human mesenchymal stem cells: a paradigm shift in regenerative therapies for intervertebral disc degeneration

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Introduction: Intervertebral disc degeneration, a leading cause of back pain, is a cell-driven process with nucleus pulposus (NP) cells from degenerate discs producing higher levels of the pro-inflammatory cytokines interleukin-1β (IL-1β) and tumour necrosis factor-α (TNFα) leading to tissue degradation. Due to the lack of currently successful treatments for disc degeneration, mesenchymal stem cell (MSC)-based therapies have been proposed, with the aim of regenerating disc tissue and restoring function. However, recent evidence suggests that MSCs may also have an anti-inflammatory or anti-catabolic role which may improve the function of resident NP cells within the degenerate IVD niche. This study aimed to investigate the anti-inflammatory effects of bone marrow-derived MSCs (BMMSCs) using a co-culture model system with human NP cells.
Materials and methods: BMMSCs (n = 5) were co-cultured in monolayer for 48 hours without contact with NP cells from either non-degenerate (n = 2) or degenerate (n = 3) discs. Co-cultures were also performed with cells exposed to 50 ng/ml IL-1β or TNFα; media without exogenous cytokine served as controls. BMMSCs and NP cells were also cultured alone, either unstimulated or stimulated with IL-1β or TNFα. Additionally NP cells were stressed for 24 hours with 50 ng/ml IL-1β or TNFα and then co-cultured with BMMSCs for 24 hours. Quantitative real-time PCR (qRT-PCR) was used to assess changes in expression of anti-inflammatory genes (IL1-Ra, TSG6, IL-10, IL-13, COX-2) by BMMSCs and anabolic genes (TGFβ, CTGF, aggrecan, versican, type II collagen) by NP cells. Western blotting was performed to quantify changes in IL-1Ra and TSG6 protein expression by BMMSCs.
Results: Co-culture of BMMSCs with degenerate NP cells (in the absence of exogenous cytokines) induced an upregulation in IL-1Ra, TSG6 and IL-10 gene expression by BMMSCs, which was not observed following co-culture with non-degenerate NP cells. Similar effects were identified when exogenous cytokines were added to BMMSCs cultured alone. Interestingly, when exogenous IL-1 and TNFα were added to the co-cultures, larger increases in expression of IL-1Ra and TSG6 were observed than were identified in either co-culture alone, or exogenous cytokine addition to BMMSCs cultured alone. Upregulation of IL1-Ra and TSG6 were confirmed at the protein level. Furthermore co-culture of BMMSCs with degenerate NP cells in this environment demonstrated significant increases in anabolic gene expression by NP cells. Stressing NP cells prior to co-culture also resulted in upregulation of the anti-inflammatory genes IL-1Ra, TSG-6 and IL-10 by BMMSCs.
Discussion and conclusions: BMMSCs exposed to a degenerate IVD-like inflammatory environment respond by increasing expression of anti-inflammatory factors and exert a positive influence on degenerate NP cell phenotype. Importantly we have demonstrated that IL-1Ra and TSG6 play significant roles in this process, suggesting that MSC implantation may result in both matrix anabolism and modulation of the inflammatory environment within the degenerate IVD niche.
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OP32
Hyaluronic acid decreases inflammatory cytokines and increases glycosaminoglycan production

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Introduction: Hyaluronic acid has been used in the clinic as an injectable biomaterial treatment for interstitial cystitis (IC). IC is a chronic debilitating disease, with a significant impact on the quality of life for patients. In IC alterations in the proteoglycan composition on the luminal wall of the bladder has been proposed as the primary pathology of IC(1). Previous studies have shown that inflammatory cytokines and glycosaminoglycan levels are drastically altered in urine(2). In order to ultimately create a comprehensive tissue engineering strategy, the mechanistic effects of HA must be investigated. In this study the role of hyaluronic acid (HA) to alter inflammatory cytokines, glycosaminoglycan pathways and permeability of urothelial cells was investigated.
Results: HA significantly decreases the TNFα induced cytokine expression in urothelial cells (Fig. 1A, 1C). Sulphated glycosaminoglycan (sGAG) expression is measured by DMMB assay shows HA significantly increases secreted sGAG (Fig. 1B). RT-PCR demonstrated that HA increases the expression of key enzymes involved in the expression of chondroitin sulphate. In order to examine the effect of HA on permeability the expression of tight junction proteins by FACS analysis and permeability using transwell migration assay was performed. The results demonstrate decreased permeability when HA is present on the cells for large molecules (Fig. 1D), with no increase in tight junction expression (Fig. 1E).
Discussion and conclusions: These results demonstrate that hyaluronic acid has an anti-inflammatory effect, increases expression of sGAG and increases permeability without altering cell surface proteins closely associated with permeability. Taken together these results show that HA has a significant effect on inflammation and GAG synthesis.
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